REMARKS

A Request for Continued Examination is filed herewith.

The following arguments are responsive to the Advisory Action mailed December 30, 2003, and support the entry of new claims 48 – 69.

I. Patentability of Pending Claims 8 - 16

Pending claims 8 - 16, which all require "permitting a population of human embryonic stem cells to form embryoid bodies" and "causing directed differentiation of said embryonic cells," are not rendered obvious in light of combining Keller (Curr. Opin. in Cell Biology, Vol. 7, pp. 862-869 (1995)) and Thomson et al. (Science, Vol. 282, pp. 1145-1147 (1998)) because there is no reasonable expectation that using the methods of Keller on human embryonic stem ("ES") cells would have been successful.

The Advisory Action of December 30, 2003 maintains that Keller in combination with Thomson renders pending claims obvious despite the arguments provided in the Applicant's response of November 14, 2003. In summary, the Action maintains that Reubinoff et al. (2000) (reference AW in the IDS submitted with the regular application on January 31, 2001) does not show that there was no reasonable expectation of success in applying Keller's methods to human embryonic stem cells ("hES cells"). A review of Reubinoff, the application, and other references, however, clearly indicates that at the time the application was filed, there was no reasonable expectation that Keller's methods would allow the formation of embryoid bodies in hES cells, a required step of the pending claims.

A. Reubinoff and Other References Show That Embryoid Body Formation Techniques for Murine ES Cells Fail when Applied to Human and Primate ES Cells

Keller reveals methods for forming embryoid bodies from *murine* ES cells. In particular, Keller notes that embryoid bodies may be formed from murine ES cells that are initially maintained in a pluripotent state on embryonic fibroblasts, or in the presence

of leukemia inhibitory factor ("LIF"). See Keller at 862. The embryoid bodies may be formed by (a) removing the murine ES cells from the presence of the feeder cells or LIF; (b) by a "hanging drop" method; or (c) placing the murine ES cells in the presence of stromal cells. See Keller at 861-862.

Reubinoff clearly indicates that such techniques would be expected to fail when applied to human ES cells. In particular, Reubinoff grew hES cells on a feeder layer. See Reubinoff section entitled "**Differentiation of human ES cells in vitro.**" The reference notes specifically that "[c]ultivation of clumps of ES cells in *hanging-drop cultures, or as aggregates on bacteriological petri dishes*, in standard medium without feeder cells resulted in considerable cell death." See id. (emphasis added). Thus, Reubinoff tried two of the methods discussed by Keller on hES cells and the methods killed the hES cells. As well, when the hES cells were cultivated to high density on a feeder layer "there was no consistent pattern of structural organization suggestive of the formation of embryoid bodies similar to those formed in mouse ES cell aggregates." See id. Thus, Reubinoff documents another technique that forms embryoid bodies in mouse ES cells, but fails to form embryoid bodies with human ES cells. These results are consistent with the findings that embryoid body formation techniques for murine ES cells also fail in marmosets and rhesus monkeys ES cells. See Applicant's Response C at 12 (discussing the failures of Thomson & Marshall and Itskovitz-Eldor et al. to form embryoid bodies using marmoset and rhesus monkey ES cells with techniques that were successful with murine ES cells).

Thus the prior art at the time of filing of the application, with regard to failed experiments done on human ES cells and other primate ES cells, clearly shows no reasonable expectation of success in forming embryoid bodies with human ES cells, a necessary step of all the pending claims. Thus, Keller and Thomson do not render the pending claims obvious.

B. The Embryoid Body Formation Techniques of the Application Differ From the Techniques of Keller

As discussed during the telephone conference, the application supports the required claim element "permitting a population of human embryonic stem cells to form

embryoid bodies" in a manner that differs from simply applying one of the embryoid body formation techniques of Keller to human ES cells. Thomson notes that human ES cells act similarly whether in the presence or absence of LIF. See Thomson at 1146, column 1. This is in contradistinction to the Keller teaching that murine ES cells will form embryoid bodies when removed from LIF, but maintain an undifferentiated, pluripotent status when the murine ES cells are in the presence of LIF. See Keller at 862. Indeed, this difference in reaction to LIF provides a motivation to develop the new protocols in the application. Thus the protocol for embryoid body formation of hES cells taught in an embodiment of the invention described in the application differs from the techniques shown in Keller. See Application at 14 – 15. For example, one protocol described indicates that "ES cells were transferred using trypsin/EDTA" before embryoid bodies are formed from the hES cells. See id. at 14. Such a step is not mentioned in Keller before embryoid body formation takes place with murine ES cells. This provides further evidence that the pending claims are not obvious from a combination of Keller and Thomson.

C. Responses to Advisory Action

The Action states that Reubinoff does not suggest that the failure to form embryoid bodies affects the response of exogenous factors. The pending claims are method claims that require the step of "form[ing] embryoid bodies." Thus if Keller and Thomson cannot teach the step of embryoid body formation with hES cells, the combination cannot render the pending method claims obvious.

As well, the application notes that embryoid body formation "allows complex signaling to occur between the cells," and thus is an important step in achieving *directed* differentiation. See Application at 8, lines 26 – 29. Indeed, the claims are not drawn to a method causing any type of differentiation of hES cells, but *directed* differentiation. See Application at line 32, page 8 through line 6, page 10 ("describ[ing] the use of 8 growth factors for *directed* differentiation of human embryonic stem cells," in an embodiment of the invention, and showing examples where the differentiation is not purely random).

The Action also suggests that since Keller teaches other methods of forming embryoid bodies with murine ES cells beyond what is mentioned by Reubinoff, an obviousness rejection may be maintained. The references presented by the Applicant, however, show that no embryoid body formation technique successful in murine ES cells was successful when applied to human ES cells. Indeed, the *reasonable* expectation, in light of all the evidence, is that murine techniques do not work with hES cells at the time of the application's filing. It cannot be argued that it would have been *reasonable* to assume that stromal cells could form embryoid bodies with hES cells when all other techniques were shown to have failed (including 2 of the 3 mentioned in Keller). As stated in MPEP § 2143.03, "at least some degree of predictability is required" for an obviousness rejection. Given the failures shown in the references, no degree of predictability exists. Indeed, assuming that the stromal cell technique would be successful is not only impermissible hindsight but even more unsupported since no presented reference, or citation in an office action, indicates that the stromal cell technique would be successful in forming embryoid bodies from hES cells as part of a method of directed differentiation at the time of filing of the application.

In no way can Keller's techniques as applied to human embryonic stem cells render the pending claims obvious, given the teachings of Reubinoff and the other references.

II. Patentability of New Claims 48 - 69

Claims 48 – 69 are added to the application. Claims 48 - 54 are drawn to directing differentiation of hES cells to human ectoderm cells; support for the claims is provided in Figure 4, at line 20, page 9 – line 6, page 10 of the application, and in other examples and embodiment of the invention. Claims 55 – 59 are drawn to directing differentiation of hES cells to human endoderm cells; support for the claims is provided in Figure 4, at line 31, page 9 – line 6, page 10 of the application, and in other examples and embodiment of the invention. Claims 60 – 69 are drawn to directing differentiation of hES cells to human mesoderm cells; support for the claims is provided in Figure 4, at

line 10, page 9 – line 6, page 10 of the application, and in other examples and embodiment of the invention.

The new claims include the steps of pending claims 8 – 16, and include further detail regarding what specific cell type(s) are human embryonic cells directed to differentiate toward, what type(s) of exogenous factors are used to direct the differentiation, or a combination of both. As such, all the new claims are patentable over the cited art for the same reasons that pending claims 8 – 16 are patentable. That is, techniques that form embryoid bodies in murine ES cells could not reasonably be expected to succeed in humans given the cited art at the time of the application's filing.

Furthermore, claims 48 - 69 are patentable even assuming, without conceding, that Keller in combination with Thomson teaches the formation of embryoid bodies from ES cells. Claims 48 - 69 are drawn to the formation of specific human cell types from human ES cells, using one or more particular exogenous factors in some claims. The cited art provides no reference that teaches the directed differentiation of human embryonic cells to the human cell types specified in claims 48 – 69.

The formation of specific cell types in some claims of the application is not shown to be formed in any of the cited art, in humans or mice. For example, claims 49 - 50, claims 53 - 54, claims 56 - 57, claim 58, and claim 60 are drawn to epidermal skin, adrenal, liver, pancreatic, and chondrocyte cells, respectively, in humans. Even if the cited art teaches the use of some factors used in some embodiments of the invention, the cited art does not teach the formation of these cell types in mice or humans.

As stated in MPEP § 2143.03, "at least some degree of predictability is required" for an obviousness rejection. Since there is no degree of predictability that the differentiation of murine ES cells is the same as human ES cells, the new claims cannot be found obvious over the cited art, regardless of whether Keller and Thomson could teach the formation of embryoid bodies by human ES cells.

III. Cancellation of Claims 1 - 7 and 17 - 47

The Applicant cancels withdrawn claims 1 – 7 and 17 – 47 without prejudice, reserving the right to pursue such claims in a follow-on continuing application.

IV. Conclusion

In view of the arguments and amendments presented, the Applicants respectfully request reconsideration of claims 8 - 16, and allowance of claims 8 - 16 and 48 – 69.

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